The response of *Escherichia coli* K12 upon exposure to hypochlorous acid and hydrogen peroxide

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Abstract

The aim of the work reported here was to investigate the growth-inhibitory activity of H0C1 and H_2O_2 toward *Escherichia coli* K12 during both logarithmic and stationary phases of the growth cycle, as well as the response of *E. coli* K12 to these oxidants. Stationary phase cultures were exposed 10 sub-inhibitory oxidising stress, and the minimum inhibitory concentrations (MIC) were determined during the ensuing 24 h. The effect of oxidant on logarithmically growing cultures was also determined. Stationary phase cultures of *E. coli* K12 responded to H_2O_2 stress, both the MIC and survival following exposure to high concentrations increasing following exposure to stress. By contrast stationary phase cells did not become more tolerant of high concentralions of HOC1 following HOC! stress. Logarithmically growing *E. coli* K12 did not display increased tolerance to either inhibitory or lethal concentrations of H_2O_2 or H0C1 following the relevant oxidising stress.

Introduction

Hypochlorous acid (HOCl) and hydrogen peroxide (H_2O_2) are oxidising bactericides used in various applications to prevent, control or decrease bacterial activity. HOCl was first employed as a wound disinfectant by Hueter in 1831, as hand disinfectant by Semmelweis in 1847, and its bactericidal activity was confirmed by Koch in 1881 (WallhauBer, 1988). HOCl is used widely as an antimicrobial agent for the control of microbial activity in recreational and industrial water systems, for sanitary applications and for surface disinfection. H_2O_2 is used amongst others in industrial water systems to control biofouling, in swimming pools and for the sanitation of surfaces and pipelines in food and other industries (Baldry and Fraser. 1988;Characklis, 1990;Cloeteetal., 1992).

Although much work on the mechanism of HOCl action in eukaryotic cells has been done, its mechanism of antibacterial action is not yet clear. HOCl is generated in white blood cells as part of the mechanism of pathogen control (Schraufstätter et al., 1990). HOCl does not enter freely into eukaryotic cells but attacks surface and plasma-membrane proteins, impairing transport of solutes and the salt balance (Schraufstatter et al., 1990). It oxidises thiol groups and inhibits plasma membrane ATPases. It appears to impair protein synthesis in cells at low concentrations for ca. 2 h following exposure, thereby affecting replication of DNA and cell division (McKenna and Davies, 1988; Schraufstatter et al., 1990). It docs not, however, cause any damage to eukaryotic genomic material. The stability and antimicrobial activity of HOCl is dependent on pH (WallhauBer, 1988). It dissociates at pH greater than 7, and the undissociateel moiety is the antibacterial agent (Hoffman et al., 1981). Above pH 7.5 it therefore loses its antibacterial activity. The antibacterial activity of chlorine dioxide and of chlorine gas in aqueous environments is also via HOCl because both react with water to form HOC! (WallhäuBer, 1988).

H_2O_2 is omnipresent in aerobic niches as it is formed, along with superoxide, as a by-product during aerobic metabolism (Fridovich. 1978). H_2O_2 reacts with a wide array of biological macromolecules such as DNA, proteins and membrane lipids (Tao et al.. 1989). For example, H_2O_2 penetrates cells, causing site-directed damage due to metal-dependant $\cdot OH$ formation (Schraufstatter et al., 1990; Storz et al., 1990). It causes DNA strand breaks and hydroxylation of bases in intact DNA. resulting in termination of replication (Schraustatteretal., 1990).Ineukaryotes H_2O_9 also inhibits mitochondrial ADP-phosphorylation (Schraustatter et al., 1990). Bacteria respond to a wide range of environmental stresses including cold, heal, osmotic pressure. UV radiation and oxidising stress. Stress responses generally lead to tolerance of cells to further exposure to otherwise lethal levels of the same stress (Vb'lker et al.. 1992; Watson, 1990). A variety of bacteria, including *Escherichia coli* (Storz et al., 1990) and *Bacillus subtilis* (Hartford and Dowds, 1992) respond to oxidising stress by producing oxidant-degrading enzymes as well as DNA-repair enzymes (Ahern, 1993; Storz et al., 1990).

The aim of the work reported here was to investigate the growth-inhibitory activity of HOCl and H_2O_2 toward *E. coli* K12 during both logarithmic and stationary phases, as well as the response of E. *coli* to these oxidants.

Materials and methods

Cultures and media used

E. coli KI2 was obtained from Prof. WOK Grabow, Dept. of Medical Virology, University of Pretoria, and was maintained on R2A agar slants (Reasoner and Geldreich, 1985) containing 1% glycerol, and subcultured monthly. R2A medium was made up as follows (per litre): 0.5 g peptone (Biolab); 0.5 g yeast extract (Biolab); 0.5 g Casamino acids (Difco); 0.5 g glucose (BDH); 0.5 g starch (BDH); 0.3 g Napyruvate (Merck); 0.3 g K_2HPO_4 (Merck); and 0.05 g $MgSO_4$ (Saarchem). For solid R2A medium. 15 gl^{-1} agar (Biolab, bacteriological grade) was added. H_2O_2 (8.8 $M\text{-}l^{-1}$) was from Saarchem. HOCl was prepared fresh as an aqueous solution by dissolving Ca(OCl), (Olin) in autoclaved deionised water.

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